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(54) Title: FABRICATION OF BIOPOLYMER PATTERNS BY MEANS OF LASER TRANSFER

(57) Abstract: A method and an apparatus for microfabricating precision patterns of biopolymers onto solid substrates by means of laser transfer are disclosed. The method involves the use of ultrafast laser pulses for transferring target biopolymer material, dimensioned according to the focal spot of the laser, from one surface of a transparent support onto the opposite positioned surface of receiving substrate. Repeating the transfer process at different target and substrate positions results in the production of extended patterns such as arrays of features or localized coatings. The apparatus is designed to produce the patters of biopolymers on solid substrates in an automated fashion. The method and the apparatus are of particular utility in producing devices for biological and biochemical assay systems such as biosensors and microarrays.

# FABRICATION OF BIOPOLYMER PATTERNS BY MEANS OF LASER TRANSFER

The present invention relates to a method and an apparatus for fabricating precise, micro-dimensioned patterns of biopolymer materials onto solid substrates, such as biopolymer coatings for biosensors or arrays of biopolymer regions for multianalyte assays, by means of laser induced transfer.

Precise deposition of biopolymers, such as proteins, nucleic acids and oligonucleotides, has been the basis for the development of various research, analytical and diagnostic applications such as biosensors, dipstick tests, protein and nucleic acid microarray chips, microfluidic assay technologies.

A variety of methods are currently available for depositing biopolymers in biosensor systems. According to most of the publication, as review by Cullen and al., (Biosensors, 3, p. 211, 1987), biopolymer materials are applied onto their binding or adsorbing substrates in solution by simple dispensing or soaking techniques. For achieving localized deposition a variety of microfluidic, gel microcasting and localized activation methods have been described, for recent examples, in Dobson et al., (EP1080367), Wagner et al., (US 6,329,209).

These publications, although they suggest solutions to the problems related to the microfabrication of biosensor systems, also demonstrate some of the limitation inherent in liquid deposition methods.

A variety of liquid methods for depositing a plurality of biopolymer samples onto solid substrates have also been published. Ordered arrays of nucleic acids and proteins samples on a porous membrane have been produced by the "dot-blot" approach, first described by Kafatos et al. (Nucleic Acids Res. 24, p.1541, 1975), and its variants. In these methods the biopolymers in solution are delivered on a retaining porous membrane by a pipette while a vacuum manifold facilitates the transfer. Dot-blot methods are unsuitable for deposition on non-porous substrates and inadequate for producing dots smaller than 1 mm.

Spots of biological samples at high densities have been produced by the use of pins or capillary dispensers that are dipped into the solution of the biological material and then used for generating spots on either porous materials or solid supports, e.g. Lehrach, et al., (Genome Analysis, Vol 1, Davis and Tilgham, Eds.,

Cold Spring Harbor Press, p. 39, 1990), and Brown and Shalon (U.S. Pat. No. 5,807,522). Arrays of pins accommodated on holders for spotting a plurality of samples and special design pins with channels generating multiple spots after single sample uptake have been disclosed by Konwallis and Raysberg (U.S. Pat. No. 6,228,659), Martinsky (U.S. Pat. No. 6,101,946) and others. Contact spotting methods using pins produce spots of variable size due to differences between the pins and variations in the rheological properties of the liquid samples. Additionally, the impact of pins on the substrate surface limits the durability of the spotting tools and may cause imperfectness of the substrate.

Wet non-contact printing methods, such us the "ink-jet" and "drop-on-demand" approaches, have also been used for spotting of biological liquid samples on various substrate surfaces, as shown by Brennan (U.S. Pat. No. 5,474,796), Tisone (U.S. Pat. No. 5,741, 554), Hayes et al., (U.S. Pat. No. 5,658,802), Theriault T.P et al., (DNA microarrays: A practical approach, M.Schena Ed., Oxford University Press, p. 100, 1999) and others. These methods are uneconomical for valuable biopolymer samples since they effectively deposit only a small fraction of the total sample loaded in the dispensing device. In addition, wet non-contact printing methods are inherently limited by the viscoelastic properties of the samples.

Alternate methods for direct synthesis of distinct oligonucleotides on solid supports have been described by Fodor, et al., (Science, 251, p. 767, 1993), Pirrung et al., (U.S. Pat. No. 5143854), Southern, et al., (Genomics, Vol. 13, p. 1008), Singh-Gasson, et al., (Nat. Biotechnol. , 17, p. 974, 1999), Quate and Stern (U.S. Pat. No. 6,271,957), Stryer et al., (U.S. Pat. 6,329,143) and others. The methods involve elaborate, combinatorial schemes of chemical synthesis at discrete regions of the solid support. These methods require relatively expensive photolithographic and/or microfluidic processing equipment and are limited to the production of relatively short oligomers.

Laser transfer based method, generally referred to as "laser induced forward transfer" (LIFT), have been used for the precise deposition of metals and their oxides, superconductors, ceramics, on solid surfaces for the microfabrication of electronic and optoelectronic devices. LIFT method utilises pulsed lasers to remove by ablating selectively material, which has been previously deposited on a laser-transparent support and transfer it onto a substrate. The receiving substrate is placed usually in parallel and at a close proximity to the thin film source under air or

vacuum conditions. Various materials have been used in LIFT applications, together with a variety of laser sources emitting from the near infrared to the ultraviolet. In most cases, transfer of material is achieved using single laser pulses, although it has also been demonstrated by means of continuous wave lasers.

The LIFT process was first shown by Bohandy et al., (J. Appl. Phys. 60, p.1538, 1986), to produce direct writing of 50 μm-wide Cu lines by using single nanosecond ArF excimer laser pulses. Fogarassy et al. (J. Mater. Res., 4, p. 1082,1989 and J. Appl. Phys., 66, p. 457, 1989) have also reported 100μm wide patterns of superconducting thin films using nanosecond ArF and Nd:YAG lasers. The deposition of diamond-like carbon films by the LIFT technique using a Cu laser and a KrF laser was also reported by Pimenov, et al., (Appl. Surf. Sci., 86, p. 208, 1995). Several other studies by Poon and Tam, (CLEO Digest, 377 1996), H. Esrom, et al. (Appl. Surf. Sci., 86, p. 202, 1995), Kantor et al., (Appl. Phys. A 54, p. 170, 1992) and Schultze and Wagner (Appl. Surf. Sci. 52, 330, 1991) have resulted in direct writing of 20μm-wide metal lines under air or helium ambient conditions also using nanosecond laser pulses. The fabrication of high resolution submicron patterns of metals and Indium Oxide complicated diffractive optics such as binary amplitude computer generated holograms by the ultraviolet femtosecond Laser Induced Forward Transfer technique have been fabricated by Koundourakis et al (Appl. Phys. Lett., 78, p. 868, 2001). The microdeposition was performed either by serial writing (pixel-by-pixel) of the diffractive pattern or by directly projecting a master hologram mask on the "target" film. Precision deposition of electrically conducting films on a working substrate has been described in ref. U.S. Pat. No. 6,159,832, Mayer F.J, by the use of an ultra-fast LIFT method. In this he reported that the use of ultra-fast laser characteristics is a perfect match for producing precision metal line "writing" on a working substrate. Laser parameters in the methods described above are not suitable for precise deposition of labile organic materials.

A direct write (DW) technique combining the laser-induced forward transfer (LIFT) method with the matrix assisted pulsed laser evaporation (MAPLE) has been demonstrated by Pique et al., (Applied Physics A 69, p. 279, 1999) and Chrisey, (U.S. pat. 6,177,151), for depositing electronics and sensor materials. The MAPLE DW technique utilises nanosecond laser pulses in a direct-write process capable of transferring materials such as metals, ceramics, and polymers onto polymeric,

metallic and ceramic substrates at room temperature. The overall writing resolution for this technique is currently of the order of 10μm. A variety of devices have been fabricated, including parallel plate and capacitors, flat inductors, conducting lines, resistors and chemoresistive gas sensors. The MAPLE DW technique always presupposes polymeric matrix material mixed with the transfer material, which under pulsed laser irradiation is more volatile than the transfer material.

The main advantage of the LIFT technique over the MAPLE DW is the simplicity of the process since it is not necessary to use any transfer matrix material. Additionally, there is some concern if the matrix preserves the properties of the transfer material and if some chemical reactions are induced during the laser irradiation.

The object underlying the present invention is to provide a method and an apparatus for producing precise, micro-dimensioned patterns of biopolymer materials on substrate surfaces at high resolution, wherein patterns include a plurality of single spaced-apart features forming arrays, and repeats of adjacent features forming localized coatings.

It is a further object of the invention to provide a widely applicable method for depositing patterns of various biopolymer materials on a variety of solid substrates, wherein the deposited biopolymers should be preferably biologically active, and an apparatus for the said method.

A further object of the invention is to provide a method and an apparatus for precisely depositing biopolymers by means of laser, wherein the biopolymer material is not damaged during the process, thus, the selection and use of materials assisting transfer is unnecessary.

Another object of the present invention is to provide said methods and equipment for microfabricating the sensing, reacting or binding surfaces of biological and biochemical assay devices.

The above outlined objects are achieved by providing the embodiments characterized in the claims.

In one aspect, the invention provides a method for microfabricating patterns of biopolymer materials on solid substrates. The method utilizes an ultrafast pulsed laser at a wavelength the biopolymer absorbs light. The target biopolymer is provided in a coating upon one surface of a laser transparent support. The receiving substrate surface is positioned at a predetermined distance from, and opposite to, the target biopolymer surface. A geometrically shaped laser pulse is subsequently projected through the support, onto the coating and at a defined location, exposing a portion of the coating material to laser energy sufficient to remove, transfer and deposit the said portion upon the receiving surface of the substrate. Transfer of biopolymer materials occurs with high precision and without damage using laser energy densities between 1 mJ/cm$^2$ and 1 000 mJ/ cm$^2$ and laser pulses with time duration between 50 femtoseconds and 50 nanoseconds, preferably in the range of from about 50 femtoseconds to about 1 nanosecond, more preferably from about 50 femtoseconds to below 1 picosecond. The method is widely applicable to proteins, nucleic acids, polysaccharides or derivatives thereof.

In another aspect the present invention provides methods for producing extended patterns of biopolymers, such as the sensing or supporting layers of biosensor systems.

In a further aspect the invention provides methods for depositing a plurality of biopolymer samples on discrete locations of the substrate, such as arrays of reagent regions for multianalyte assays.

Further, an apparatus for producing patters of biopolymers on solid substrates in an automated fashion is described.

The methods and the apparatus are of particular utility in producing devices for biological and biochemical assay systems such as biosensors and microarrays.

An advantage of the present invention is the precision and uniformity of the deposition method. The use of the ultrafast laser pulses for biopolymer transfer propels materials with low angular divergence, avoids splattering and vaporization, thus, produces features with minimum spread and high spatial resolution.

A further advantage of using ultrafast laser pulses is that minimizes the adverse thermal effects of the process and lowers the required energy threshold for transfer,

thus, thermally labile biopolymer materials are not damaged during transfer, particularly when using a pulse width of about 1 nanosecond or below, preferably below 1 picosecond.

Another advantage of the present invention is the simplicity of the method wherein biopolymers absorb the energy of the ultrafast laser pulses and can be deposited on the substrates without the assistance of any transferring matrix material. Compared to the lithographic methods this method is a "clean", one-step process and it is not limited to oligomer structures only.

Another advantage of the present invention is versatility of the method that can be easily adapted and used for depositing a wide variety of biopolymer materials onto various receiving substrates.

Figure 1 is a schematic view of an apparatus, embodiment of the present invention. Sequential events of the deposition process are also illustrated, for clarity.

The apparatus schematically illustrated in Figure 1, comprises a laser source 1 generating ultrafast pulses, an attenuator 2 adjusting the energy density, a laser beam modulator 3, an aperture 4 for shaping the focal spot of the laser beam, a mirror 6 at the laser wavelength for directing the beam towards the target, an objective lens 7 for focusing the beam onto the target material 10 coated on the lower surface of a laser transparent target holder 9, a target holder 8 (holding a plurality of targets in this schematic illustration), a substrate platform 11 for holding and positioning the receiving substrate 12, translation stages controlled by drivers 13 for moving the target holder 8 (in the xy plane) and the substrate platform 11 (in the x'y' plane), a computer system 14 coordinating the pulses of the laser and the movement of the translation stages. A CCD camera 5 can also be included for monitoring the deposition process.

In the following preferred embodiments the present invention is outlined in more detail.

The method is useful for depositing by laser induced transfer a variety of biopolymers that absorb light at the corresponding wavelength of the laser. Transfer biopolymers include, but are not limited to, polypeptides and proteins such as enzymes, antibodies, antigens, protein A, hormones, receptors, lectins, avidin, oligopeptides, nucleic acids such as DNA, RNA, oligonucleotides, polysaccharides, glycoproteins, proteoglycans, glycolipids, lipids, obtained from either biological sources or by chemical synthesis, derivatives thereof, and artificial counterparts such as the peptide nucleic acids.

Prior to transfer the biopolymer is applied onto the lower surface of the target support. Most biopolymers are soluble in aqueous solutions. Other volatile solvents including, but not limited, to ethanol, acetone, diethylether, chloroform, and their mixtures, can also be used if necessary to obtain the biopolymer in solution of suspension. The transfer biopolymer is applied onto the surface of the target holder in solution, optionally, with a variety of compounds selected from the following functional groups:

Buffers such as carbonate, formate, acetate, citrate, phosphate, borate, dimethylarsinate, ethanolamine, triethanolamine, trimethylamine, triethylamine, imidazole, histidine, pyridine, collidine tris(hydroxymethyl)aminomethane, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 2-(N-morpholino)ethanesulphonic acid, 1,3-bis[tris (hydroxymethyl) methylamino]propane, 3-(N-morpholino)-2-hydroxypropane-sulfonic acid, 1,4-piperazinediethanesulfonic acid, at concentrations of 10-100 mM, to maintain the pH of the solution.

Detergents such as dodecyl sulfate, lauroyl sarcosine, deoxycholate, sulfosalicylate, diiodosalicylate, cetyldimethylethylammonium bromide, 3-[3-cholamidopropyl9-dimethylammonio]-1-propane sulfonate, alkyl-glucosides, alkyl-thioglucosides, alkyl-maltosides, alkyl-thiomaltosides, polyoxyethylene esters, polyoxyethylene ethers, polyoxyethelenesorbitan esters, alkyl-N-hydroxyethylglucamides, at 0,1-10 % weight per volume, to solubilize insoluble biopolymers.

Chaotropic agents such trichloroacetic acid, perchlorate, urea, guanidine hydrochloride, guanidine thiocyanate, formamide, glyoxal, used at concentations 0,5-5 M, when the biopolymers are intended denatured.

Reductants such as dithiothreitol, dithioerythritol, β-mercaptoethanol, at a concentaration range 5-50 mM, to prevent oxidation of thiols.

Chelating agents such as ethylenediamine tetraacetic acid, ethyleneglycobis(β-aminoethyl)ether tertaacetic, at 1-20 mM acid to bind unwanted bivalent metals.

Stabilizers such as glycerol and other polyols, glucose, N-acetyl glucosamine, sorbitol, ascorbic acid, sucrose, trehalose, at concentration range 5-20 % weight per volume, to maintain biological activity and conformation of the biopolymers.

Polymers able to imbibe or retain water, generally referred to as hygrogels, such as agarose, alginates, dextran, poly(ethyleneglycol), polyethylenimine, polyacrylic acid, polyacrylamide, poly(1-vinyl-2-pyrrolidon), poly(hydroxyethyl-methacrylate), poly{[tris(hydroxymethyl)-methyl]acrylate}, usually at a concentration range 1-10% weight per volume, to provide an aqueous microenvironment to biopolymers.

Inorganic salts such as sodium chloride, ammonium sulfate, and organic compounds and solvents such as dimethylsulphoxide, to modulate the ionic strength of the solution and the solubility of the biopolymers.

Specific enzyme inhibitors such as (4-amidinophenyl)methanesulfonyl fluoride, leupeptin, 3,4-dichloroisocoumarin, N-[N-(L-3-trans-carboxirane-2-carbonyl)-L-leucyl]-agmatine, phenylmethylsulfonyl fluoride, N-ethylmaleimide, benzamidine for proteases, ethylenediamine tetraacetic acid for deoxynucleases, typically at concentrations 0.1-1 mM, to protect enzyme degradable biopolymers.

Preservatives such as sodium azide, methylisothiazone, 2-[(ethylmercurio)thio]benzoic acid, bromonitrodioxane, at concentrations 0,01-0,1% weight per volume, to protect biodegradable biopolymers.

Dyes such as 1-anilinonaphthalene-8-sulfonic acid, 3-hydroxy-4-[2-sulfo-4-(4-sulfophenylazo)phenylazo-2,7-naphthalenedisulfonic acid, 2,7-diamino-10-ethyl-phenyl-phenanthridinium bromide, to facilitate the monitoring of the coating and deposition process.

Typically, the above compounds are selected with regard to the function they serve and their compatibility with the receiving substrates. For example, if a biopolymer sensitive to drying, is to be transferred and immobilized via its amine groups on an activated substrate, the hydrogel of choice should not possess any competing amine groups.

The biopolymer solution is applied on the lower surface of the support to form a uniform coating by a variety of techniques such as dispensing, spin-on-disk, spraying, followed by evaporation of the solvent. The working concentration of the biopolymer in the solution depends on the solubility of the biopolymer, the viscosity of the solution, the hydrophobicity of the support, and the technique used for coating. Single or multiple applications can be used and the final thickness of the biopolymer coating can be between 100nm and 10 μm.

The target support should be of high optical and surface quality and composed of a material that does not absorb at the wavelength in use. Materials such as fused silica, sapphire, magnesium and calcium fluoride can be used in a wide range of wavelengths from UV to IR.

Generally, the efficiency of the laser light coupling into the materials depends on the materials optical properties, the wavelength, and the time duration of the incident light. Particularly, the laser wavelength source is selected with regard to the absorption spectrum of the biopolymer and preferably with the shortest pulse duration available in order to minimize the thermal effects of the process and prevent damage of the deposited material. A variety of pulsed laser sources are available in the full spectral range from UV to IR and can be utilized in this method including, but not limited to, excimer lasers at wavelengths 248 nm (KrF) and 308 nm (XeCl) with pulse duration 30 ns and pulse repetition frequency up to 100 Hz, excimer laser at wavelength 248 nm (KrF) with pulse duration 0.5 ps and pulse repetition frequency up to 10 Hz, Nd:YAG and Nd:Glass lasers at wavelengths 1064 nm, 532 nm, 355 nm, 266 nm with pulse duration 6 ns and 0.5 ps and pulse repetition frequency up to 10 Hz and Ti:sapphire laser at wavelength 800 nm, and 400 nm with pulse duration 150 fs and pulse repetition frequency up to 1000 Hz.

The pulsed laser beam is projected onto the target material by any means known in the art of laser optics including but not limited to mirrors, refractive and reflective lenses.

The pulsed laser energy can be adjusted by means of an attenuator in order to obtain energy densities above the transfer energy threshold and below an energy density of 1000 mJ/cm$^2$ to avoid any damage or vaporization of the biopolymer material.

The pulsed laser beam shape and dimensions onto the target material can be adjusted by means of a variable aperture with any special shape such as, rectangular or triangular or circular etc. in order to expose the target biopolymer material in an area so that, a precise and defined portion of the material is transferred onto the substrate.

In order to obtain spatial resolution between 1 and 500 μm an optical system is projecting the aperture projection on a large-reduction basis onto the target material.

The receiving substrate should be placed in parallel and in close proximity with the target substrate. Typically, the distance between the lower side of the target material and the upper side of the receiving substrate is varied from 1 μm to 500 μm with 1 μm resolution.

The pulsed laser beam, the target material and the substrate can be positioned in relation to each other and can be controlled and moved with respect to each other by means of translation stages and computer controlled translation stage drivers. This is a well-known technology in the field of laser micromachining i.e. laser cutting, drilling etc.

More specifically, the laser beam is directed onto the target material and irradiates the target material with sufficient energy to remove and transfer a selected portion of the biopolymer material onto the substrate. Repeating the transfer process at different target and substrate position i.e. pixel by pixel step and repeat operation by means of a PC and the translation stages driver results in the production of patterns such as a plurality of single spaced apart features, forming arrays, and repeat of adjacent features, forming localized coatings. Furthermore, a plurality of targets with different biopolymer materials could be used resulting in a plurality of distinct deposits.

During the transfer process, the working area could be monitored through an imaging system, including but not limited to a CCD camera and an optical microscope.

A wide variety of materials can be used as receiving substrates. They can be any solid fibrous or porous, preferably planar, material including, but not limited to, glass, silicon, metals, polystyrene, nylon, polyacrylamide, polyester, cellulose, dextran, agarose, or a derivative thereof, if necessary chemically treated or properly coated in order to bind the deposited biopolymers.

The present invention will be further explained with reference to the following examples that are specific applications indented to illustrate, but in no way to restrict, the present application.

EXAMPLE 1

In this specific application, the protein Ribonuclease I is transferred at defined positions on a sensochip used for surface plasmon resonance measurements. The device produced is used for studying the binding of the Human Placental Ribonuclease Inhibitor to the immobilized Ribonuclease I.

To prepare the target material 20 mg of Bovine Pancreas Ribonuclease were dissolved in 330 µl of an aqueous solution containing 100 mM ammonium formate at pH 8,10 % (v/v) methanol, 2,5 % (w/v) trehalose and 0.05 % (w/v) agarose and then applied to form a coating on the surface of a fused silica disk (25.4 mm diameter and 1mm thick), by the spin on disk technique (at 250 rpm, for 20 sec). The coating was left overnight to dry at ambient temperature in a ventilated fume hood.

The apparatus used for transferring the protein material utilized a Nd:Glass pulsed laser operating at 266 nm with pulse duration 0.5 picosecond. The laser beam was focused through a high power image projection micro-machining system based on the inverse microscope principle. The system was performing mask projection on a large-reduction basis (X30) onto the target. The estimated depth of focus was 2µm and the laser spot size could be varied between 1 and 250 µm. The energy density of the laser could be adjusted by means of the attenuator at values between 50mJ/cm$^2$ and 550mJ/cm$^2$. During the deposition process the target area could be viewed through an imaging system including a CCD camera and microscope lenses.

The fused silica disk was placed opposite to the sensochip surface so that the distance between the protein coating and the activated dextran coating was 20 µm. The laser beam was focused onto the coating of the target protein, the energy density was adjusted at 50 mJ/cm$^2$ and the laser beam geometrical shape was a rectangular spot of 250 µm x 250 µm. The irradiated coating material was ejected and deposited onto the dextran coating of the substrate. Each laser shot resulted

into an approximately 250 µm square spot of 1µm thick. The transfer process was repeated at different target and substrate locations by means of computer controlled translation stages in order to form two uniformly coated regions of 2mm X 2mm at positions corresponding to the openings of the microfluidic system used for further treatment and analysis.

EXAMPLE 2

In this specific application, distinct DNA samples are transferred onto nitrocellulose coated microscope slides. The device produced is used for gene expression profiling by hybridization methods.

The double-stranded DNA samples obtained by the polymerase chain reaction method were solubilized at 3-5 mg/ml concentration in a solution containing 10 mM sodium citrate pH 8, 15mM sodium chloride, 1mM EDTA, and 0.05 % (w/v) agarose for facilitating coating. The solutions of the DNA samples were applied on the fused silica disks (25.4 mm diameter and 1 mm thick) by a pipette and were left to dry at a ventilated fume hood to form a coating of approximately 0.5 µm thick. The laser transparent support was irradiated from the back side using a KrF pulsed excimer laser operating at 248 nm with pulse duration 0.5 ps. The laser energy density was adjusted at 100 mJ/cm$^2$ onto the coating and the spot was was 100 µm x 100 µm. The irradiated coating material was ejected and deposited onto the nitrocellulose coated glass substrate. The distance between the targets and the substrate was adjusted at 20 µm. Each laser shot resulted into an approximately 200 µm square spot of 0.5µm thick and different DNA samples was deposited at discrete substrate regions on the microscope slide.

## Claims

1. A method for fabricating patterns of biopolymer materials on solid substrates comprising the steps of
   (a) providing an ultrafast pulsed laser at a wavelength the biopolymer absorbs light,
   (b) providing a target coating comprising the biopolymer upon one surface of a laser transparent support,
   (c) providing a receiving substrate,
   (d) positioning the receiving substrate surface at a predetermined distance from, and opposite to, the target coating surface,
   (e) projecting a geometrically shaped laser pulse through the support onto the coating, at a defined location, exposing a portion of the coating material to laser energy sufficient to remove, transfer and deposit the said portion upon the receiving surface of the substrate, wherein the duration of the laser pulse is in the range of from about 50 femtoseconds to below 1 picosecond.

2. The method according to claim 1, wherein the energy density of the laser is between 1 mJ/cm$^2$ and 1 000 mJ/ cm$^2$

3. The method according to claim 1 or 2, wherein the biopolymer is selected from the group consisting of proteins, polypeptides, oligopeptides, nucleic acids, DNA, RNA, oligonucleotides, polysaccharides, oligosacharides, glycoproteins, proteoglycans, antigens, antibodies, receptors, lectins, enzymes or derivatives thereof.

4. The method according to anyone of claims 1 to 3, wherein the deposition step is further repeated at different locations on the target coating and the substrate is moved so as successive deposits of the material are at overlapping, or adjacent, or space-apart, spatial relation on the receiving surface.

5. The method according to anyone of claims 1 to 4, wherein a plurality of target coatings are provided, each comprising a distinct biopolymer sample, so as each biopolymer sample is deposited at a defined and discrete region on the substrate.

6. An array of discrete reagent regions obtainable by the method of claim 5.

7. A biosensor system comprising a sensing region obtainable by the method of any one of the claims 1 to 5.

8. A biosensor system comprising an immobilization matrix region obtainable by the method of any one of the claims 1 to 5.

9. An apparatus for producing patterns of biopolymers on solid substrates comprising
   (a) an ultrafast pulsed laser source,
   (b) an optical delivery system,
   (c) a holder for target supports,
   (d) translation stage moving target holder,
   (e) a receiving substrate platform,
   (f) translation stage moving substrate platform,
   (g) microcomputer system controlling the translation stages and the laser.

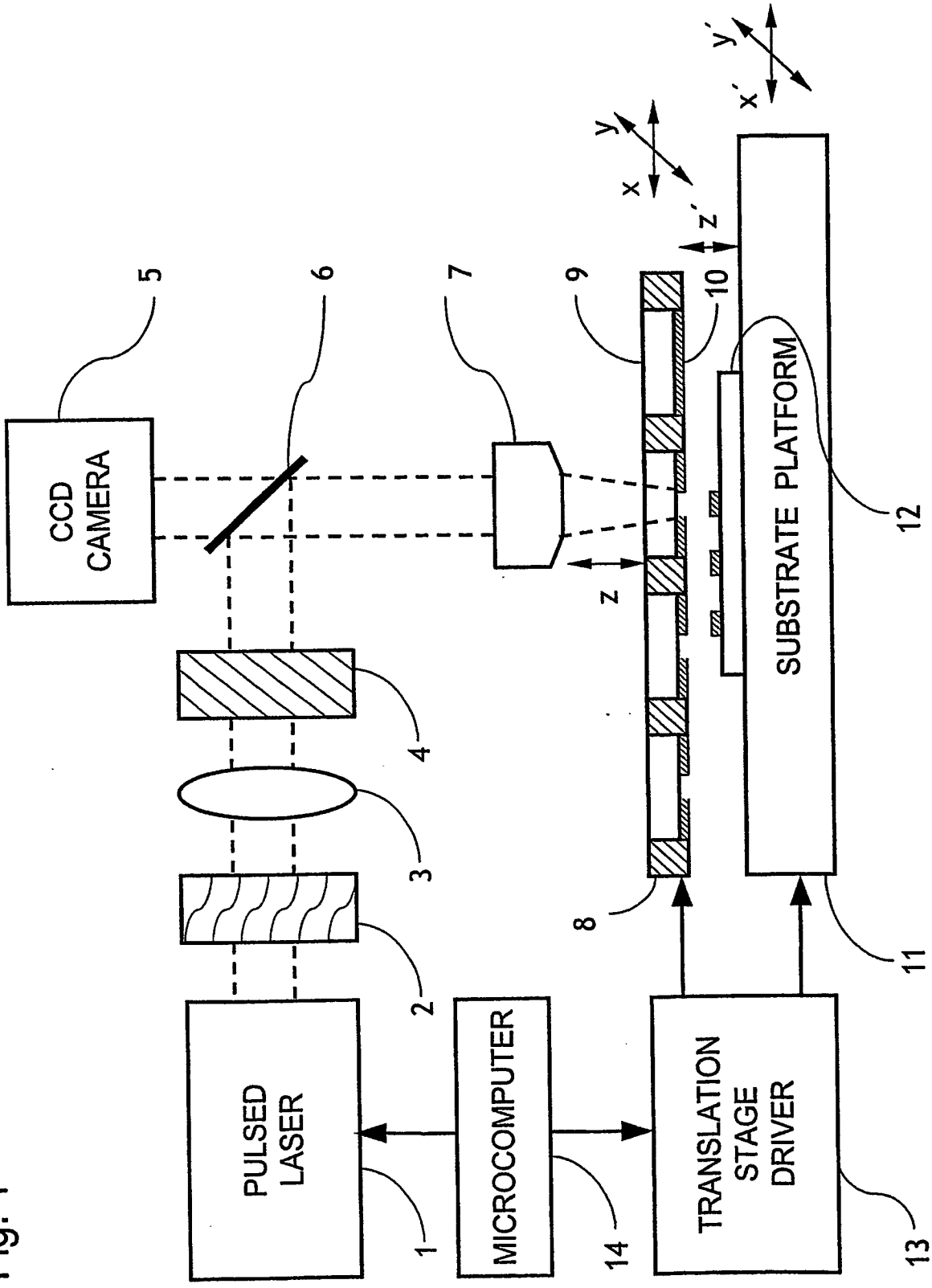


Fig. 1

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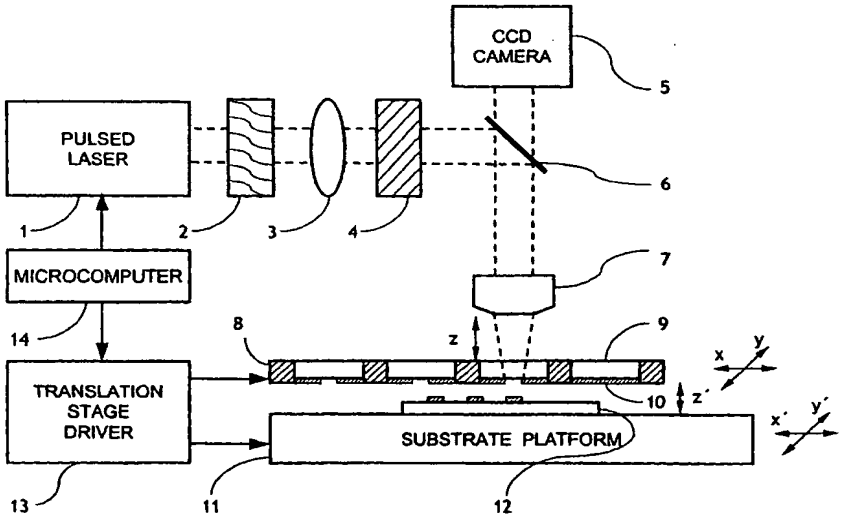
*[Continued on next page]*

(54) Title: FABRICATION OF BIOPOLYMER PATTERNS BY MEANS OF LASER TRANSFER




(57) Abstract: A method and an apparatus for microfabricating precision patterns of biopolymers onto solid substrates by means of laser transfer are disclosed. The method involves the use of ultrafast laser pulses for transferring target biopolymer material, dimensioned according to the focal spot of the laser, from one surface of a transparent support onto the opposite positioned surface of receiving substrate. Repeating the transfer process at different target and substrate positions results in the production of extended patterns such as arrays of features or localized coatings. The apparatus is designed to produce the patters of biopolymers on solid substrates in an automated fashion. The method and the apparatus are of particular utility in producing devices for biological and biochemical assay systems such as biosensors and microarrays.

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# INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/14761

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01J G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, INSPEC, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| X | P. K. WU ET AL.: "The deposition, structure, pattern deposition, and activity of biomaterial thin-films by matrix-assisted pulsed-laser evaporation (MAPLE) and MAPLE direct write" THIN SOLID FILMS, vol. 398-399, November 2001 (2001-11), pages 607-614, XP004328744 elsevier, nl ISSN: 0040-6090 the whole document<br>---<br>-/-- | 1-9 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 3 July 2003 | 10/07/2003 |

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|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Veefkind, V |

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International Application No
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